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Barley spikelet morphometry analysis

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Transcriptional landscapes of floral meristems in barley

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Detailed protocol

Barley spikelet morphometry analysis

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The lateral spikelet morphometry analysis was conducted for the barley *dense and erect panicle 1* (*dep1*) mutant alleles, BW-*ari-e.1*, BW-*ari-e.GP*, *ari-e.119,* their respective wild type progenitors Bowman (BW-*ari-e.1*, BW-*ari-e.GP*) and Foma (*ari-e.119*). Similarly, the lateral spikelet morphometry analysis was conducted for barley *uniculm 4* (*cul4*) mutants *cul4.24* (wild type – Flare), and BW-*cul4.5* (wild type – Bowman) and RNA guided endonuclease knockout (RGEN KOs) lines of barley*SPL14*and azygous Golden Promise. Based on our transcriptomic and phenotypic analyses, we found that wild-type CUL4 and SPL14 act as negative regulators of barley lateral spikelet development (Thiel et al. 2021). However, in the mutants of*cul4*, the lateral spikelet development is promoted (increase in the size of floral organs such as lemma, palea, stamens, and carpels; Thiel et al. 2021), including awn elongation and grain development in the lateral spikelets of barley *SPL14* RGEN KOs (Thiel et al. 2021). Our transcriptomic and phenotypic analysis revealed barley *DEP1* as a positive regulator of lateral spikelet development, while the mutants o f *dep1* showed a reduction in lateral spikelet size (Thiel et al. 2021). To analyze the developmental promotion and growth of lateral spikelets in *cul4* mutants and reduction in the lateral spikelet size of *dep1* mutants, we quantified the morphometry parameters, such as spikelet length, width, and area by imaging and analysis of lateral spikelets in the respective mutants and their wild type progenitors. In general, the grain morphometry parameters in cereals can be assessed by digital Marvin seed analyzer (GTA Sensorik GmbH, Neubrandenburg, Germany). However, the lateral spikelets of both wild-type and mutants of *cul4* and *dep1* do not set grains, making it not possible to analyze these parameters using Marvin. Hence, an image analysis method was used to extract lateral spikelet morphometry parameters of *cul4*, and *dep1* mutants. The step-by-step procedure followed for this analysis is described below.

1. Harvest green spikes from the main culm after completion of grain filling and onset of senescence (senescence of spikelet starts at the tip and is indicative of growth completion)
2. Excise lateral spikelets from the well-developed portion of the spike using forceps (boxed area in the **fig. 1a**; in our study we analyzed lateral spikelets from spikelet nodes 8 to 17 [(base to top); 10 lateral spikelets]
3. Image the excised lateral spikelets under a stereomicroscope (Zeiss Stemi-2000) using appropriate magnification and scaling factors.
4. Import the acquired images into Axiovision image analysis software (Zeiss Microscopy GmbH)
5. From the measure tab of Axiovision, select the outline option, and draw contours of the spikelets (**fig.1b**). To conclude the contour, click the right mouse button. The contour can be adjusted by dragging the correction points spread across the contour.
6. Select the “create data table” option from the measure tab to export the contour information such as area (μm^2), bound width (µm), and bound height (µm).
7. Compare the lateral spikelet area, height, and width, in respective mutant and wild type pairs and conduct a significance test with two-tailed *Students t-test* in MS-office Excel.

The central spikelet morphometry parameters such as length, width, and area were also analyzed for *dep1*, *cul4* mutants, and their respective wild types. Since the central spikelets set grains in both wild type and mutant spikes of *dep1*, and *cul4*, we acquired the quantification data using the Marvin seed analyzer.
Note: the barley lateral spikelets are pedicellated. While excising the lateral spikelets using forceps, it is possible to damage part of the pedicel or a portion of the pedicel is still attached to the main spike after lateral spikelet excision. For this reason, we excluded pedicel while drawing contours of lateral spikelets (**fig. 1b**). The glumes of lateral spikelets were clipped off before imaging.

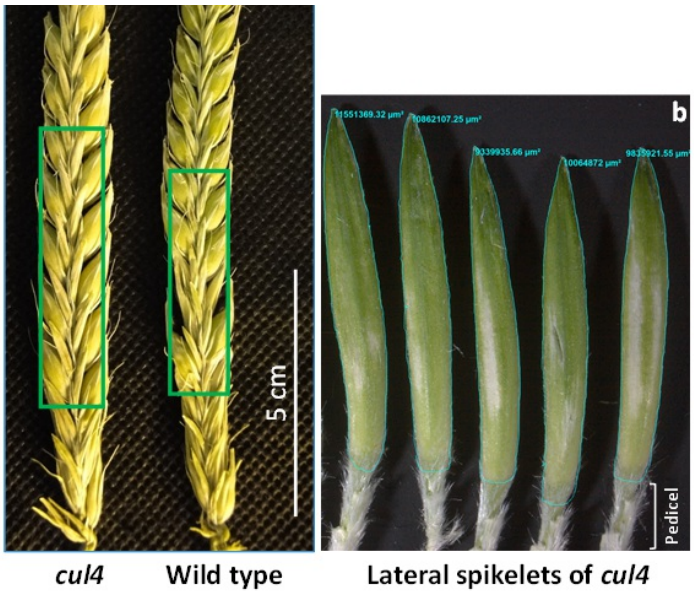

Figure 1. Lateral spikelet morphometry analysis
(a) Representative spikes of *cul4* mutant and wild type. The boxed area in green indicates the area from where the lateral spikelets are excised for imaging and morphometry analysis. (b) lateral spikelets with contours drawn around the spikelets.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Koppolu, R. (2021). Barley spikelet morphometry analysis. Bio-protocol Preprint. bio-protocol.org/prep1075.
2. Thiel, J., Koppolu, R., Trautewig, C., Hertig, C., Kale, S. M., Erbe, S., Mascher, M., Himmelbach, A., Rutten, T., Esteban, E., Pasha, A., Kumlehn, J., Provart, N. J., Vanderauwera, S., Frohberg, C. and Schnurbusch, T.(2021). Transcriptional landscapes of floral meristems in barley. Science Advances 7(18). DOI: 10.1126/sciadv.abf0832